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ACUTE ORAL TOXICITY OF DMSO (DIMETHYL SULFOXIDE) 1/)

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INSTITUTE REPORT NO. 164

**ACUTE ORAL TOXICITY OF DMSO PROCESS STREAM SAMPLES
IN MALE AND FEMALE RATS**

**CRAIG W. WHITE, DVM, CPT VC
JUSTO RODRIGUEZ, BS, SP4
and
GLEN E. MARRS, JR, DVM, MS, MAJ VC**

**TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT**

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Toxicology Series 64

**LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129**

Acute Oral Toxicity of DMSO Process Stream Samples in Male and Female Rats (Toxicology Series 64)--White, Rodriguez and Marrs

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The acute toxicities of a single oral dose of the DMSO process stream samples, DMSO Evaporator Sludge, DMSO Recycle Solvent, and Virgin DMSO, were determined in male and female albino Sprague-Dawley rats. The DMSO Evaporator Sludge and Virgin DMSO solutions produced no deaths in male or female rats at a limit dose of 5.0 ml/kg. The DMSO Recycle Solvent was more toxic, and a LD ₅₀ with 95 percent confidence limit was calculated by probit analysis. The DMSO Recycle Solvent LD ₅₀ was 2.1 ml/kg in male rats, 95 percent confidence limit		

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(1.5 ml/kg, 2.8 ml/kg), and 1.3 ml/kg in female rats, 95 percent confidence limit (1.0 ml/kg, 1.8 ml/kg).

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ABSTRACT

The acute toxicities of a single oral dose of the DMSO process stream samples, DMSO Evaporator Sludge, DMSO Recycle Solvent, and Virgin DMSO, were determined in male and female albino Sprague-Dawley rats. The DMSO Evaporator Sludge and Virgin DMSO solutions produced no deaths in male or female rats at a limit dose level of 5.0 ml/kg. The DMSO Recycle Solvent was more toxic, and a LD₅₀ with 95 percent confidence limit was calculated by probit analysis. The DMSO Recycle Solvent LD₅₀ was 2.1 ml/kg in male rats, 95 percent confidence limit (1.5 ml/kg, 2.8 ml/kg), and 1.3 ml/kg in female rats, 95 percent confidence limit (1.0 ml/kg, 1.8 ml/kg).

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PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Division of Research Support
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, MD 21701

PROJECT/WORK UNIT/APC: USAMBRDL DMSO Process Stream Samples Project,
APC TLO1

GLP STUDY NUMBERS: 82039 and 83004

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC
Diplomate, American College of
Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Craig W. White, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP4 Justo Rodriguez, BS

PATHOLOGIST: MAJ Glen E. Marrs, Jr., DVM, MS, VC
Diplomate, American College of
Veterinary Pathologists.

STATISTICIAN: Virginia L. Gildengorin, PhD

REPORT AND DATA MANAGER: Carolyn M. Lewis, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, study
protocols, raw data, retired SOPs, and an
aliquot of the test compound will be
retained in the LAIR Archives as GLP
Studies 82039 and 83004

TEST SUBSTANCE: DMSO Process Stream Samples:

Recycle Solvent (TP013)
Virgin DMSO (TP014)
Evaporator Sludge (TP015)

INCLUSIVE STUDY DATES: 29 December 1982 - 19 January 1983 (82039)
16 March 1983 - 5 April 1983 (83004)

OBJECTIVE: To determine the acute oral toxicity of DMSO Process
Stream Samples: Recycle Solvent (LAIR Code TP013), Virgin
DMSO (LAIR Code TP014), and Evaporator Sludge (LAIR Code
TP015) - in male and female Sprague-Dawley rats.

ACKNOWLEDGEMENTS

The authors wish to thank SP5 Leonard Sauers, MS; SP4 Lawrence Mullen, BS; SP4 Thomas Kellner, BA; and SP4 Evelyn Zimmerman for assistance in performing this research. Additionally, the authors wish to thank Dr. Jack Dacre and CPT James Carroll of the U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD 21701 for consultation services.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study numbers 82039 and 83004 described in this report to be scientifically sound and the results in this report and interpretations to be valid. The studies were conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

John T. Froin 1 July 83
JOHN T. FROIN / DATE
COL, VC
Study Director

Justo Rodriguez 1 July 83
JUSTO RODRIQUEZ, BS / DATE
SP4, USA
Co-Principal Investigator

Glen E. Marrs, Jr. 1 July 83
GLEN E. MARRS / DATE
MAJ, VC
Pathologist

Carolyn M. Lewis 1 July 83
CAROLYN M. LEWIS, MS / DATE
DAC
Data Manager

Craig W. White 1 July 83
CRAIG W. WHITE / DATE
CPT, VC
Principal Investigator

Virginia B. Gildengorin 5 July 83
VIRGINIA B. GILDENGORIN, PhD / DATE
DAC
Statistician



DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

IN REPLY TO
ATTENTION OF:

SGRD-UJLZ-QA

25 Jul 83

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82039 the following inspections were made:

5 Jan 83 - 0900
5 Jan 83 - 1030
19 Jan 83

The report and raw data for this study were audited on 25 Jul 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Apr 83 report to management and the Study Director.

I hereby certify that in relation to LAIR GLP study 83004 the following inspections were made:

18 Mar 83
5 Apr 83

The report and raw data for this study were audited on 25 Jul 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Apr 83 and Jul 83 report to management and the Study Director.



NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

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Acute Oral Toxicity Of DMSO Process Stream Samples In Male and Female Rats--White et al

Dimethyl sulfoxide (DMSO) is a widely used industrial solvent/reagent which is currently being considered for use by the Holston Army Ammunition Plant as a replacement process solvent in the recrystallization phase of HMX/RDX production. A pilot recrystallization facility was established and put into small scale operation to evaluate the projected advantages of DMSO as a process stream solvent. Samples of the DMSO process stream were taken from two locations at the recrystallization facility. The solutions collected were designated DMSO Recycle Solvent and DMSO Evaporator Sludge. The industrial grade DMSO, also sampled, was designated Virgin DMSO. The Process Stream Samples, analyzed by the Holston Defense Corporation Laboratory, contained major and minor cyclic and noncyclic nitramines. Since nitramines are neurotoxic, their presence in the samples represented a potential health hazard to workers exposed during this production process. Thus, before full-scale DMSO process solvent production can begin, a complete health hazard assessment including delineation of the acute toxicity of the DMSO solutions is required (1-4).

The Toxicology Group, Letterman Army Institute of Research, was assigned by the U.S. Army Medical Research and Development Command the task of performing a major part of the Phase I Toxicity Testing. During Phase I, data will be gathered which are pertinent to the determination of potential toxicity associated with worker exposure to DMSO process streams. These initial toxicity assessments will be used to develop the appropriate exposure criteria and/or worker protection requirements.

Objective of the Study

The objective of this study was to determine the acute oral toxicity in male and female Sprague-Dawley rats of the DMSO process stream samples: Recycle Solvent (LAIR Code TP013), Virgin DMSO (LAIR Code TP014), and Evaporator Sludge (LAIR Code TP015).

METHODS

Test Substance

1. Chemical name: DMSO Recycle solvent (TP013)
2. Chemical name: Virgin DMSO (TP014)
3. Chemical name: DMSO Evaporator Sludge (TP015)

Identification of nitramine impurities in the test samples by high pressure liquid chromatography (HPLC) was performed by the Holston Defense Corporation. Results from these analyses appear in Appendix A. The samples were three years old at the time the study was conducted, thus analyses for chemical stability was not performed.

Animal Data

A combined total of 166 male and female Sprague-Dawley rats were obtained from Banton Kingman, Fremont, CA 94538. Appendix B contains additional animal data.

Environmental Conditions

A commercially available certified rodent ration and tap water were provided ad libitum to the animals during this study. A complete listing of the environmental conditions of this study appears in Appendix C.

Dosing

Limit Test (GLP Study Number 82039)

Four dose groups were selected at random, consisting of seven male and seven female rats per group. The Recycle Solvent, Evaporator Sludge, Virgin DMSO, and Vehicle Control (reagent grade DMSO) were dosed at a limit level of 5.0 ml/kg (5). The cage control group (4 males, 7 females) was untreated. No dilution of any of the dosing materials was made. The DMSO materials were heated to 40 C to optimize solute/solvent interactions at dosing (as requested by the sponsor). The volumes administered ranged from 0.84 ml to 1.2 ml.

All animals received a single dose of the appropriate sample on 5 January 1983. Sterile, disposable, 3 ml syringes (Becton, Dickinson & Co., Rutherford, NJ) fitted with 16 gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized for oral administration of the samples. The dosing procedures were conducted without animal sedation or anesthesia.

Recycle Solvent LD₅₀ Test (GLP Study Number 83004)

Six test groups were selected at random, consisting of eight male and eight female animals per group. Four dose levels were selected (0.8, 1.6, 3.1, and 4.7 ml/kg) based upon the fact that a 100 percent mortality occurred at the limit dose of 5.0 ml/kg. The Recycle Solvent was not diluted before dosing. The vehicle control animals received 1.0 ml of reagent grade DMSO per animal. The dose volumes ranged from 0.15 to 1.19 ml. The Recycle Solvent and the Vehicle Control were heated to 40 C to optimize the solvent/solute interaction at dosing (as requested by the sponsor).

All animals received a single dose of the recycle solvent on 23 March 1983. Sterile disposable syringes (Becton, Dickinson & Co., Rutherford, NJ) fitted with 16 gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized for the oral administration of the samples. The dosing procedure was conducted without animal sedation or anesthesia.

Observations

The animals were observed for mortality and signs of acute toxicity throughout the dosing procedure and intermittently for 1-2 hours after dosing and during the afternoon on the day of dosing. Observations were also conducted daily for the remainder of the study. Body weights were recorded before dosing and twice weekly until death or termination of study. Appendix D contains a complete listing of observation periods.

Statistical Methods

Statistical analyses were performed on the Recycle Solvent and LD₅₀ study data. The LD₁, LD₅₀, LD₉₅ were derived by Bliss probit analysis, as described by Finney (6).

Duration of the Study

The study period for the Limit Study (82039) was 15 days with a 7-day quarantine/acclimation period before the study. The study period for the LD₅₀ Study (83004) was 14 days with a 7-day quarantine/acclimation period. Appendix D is a listing of historical events for the two studies.

Changes to Original Procedures

When GLP Study 82039 was originally designed, it was decided that the "Virgin" DMSO group would serve as the vehicle control group. The Study Director determined that the Virgin DMSO group should be in a dose study group and that the vehicle control should be reagent or laboratory grade DMSO. Group 1 was designed as the vehicle control group rather than the untreated control group. Four unassigned male and seven unassigned female rats were then designated as the untreated control group or cage control group.

RESULTS

Mortality

Table 1 lists the compound related deaths by group and the percent mortality for the Limit Test, GLP Study 82039.

Table 1

Compound Related Deaths by Group
Limit Test, 82039

Group	DMSO Process Stream Samples	Compound Related Deaths/No. in Group		Percent Mortality	
		Male	Female	Male	Female
1	Vehicle Control (Reagent Grade DMSO)	0/7	0/7	0	0
2	DMSO Recycle Solvent TP013	7/7	7/7	100	100
3	Virgin DMSO (Industrial Grade DMSO) TP014	0/7	0/7	0	0
4	Evaporator Sludge TP015	0/7	0/7	0	0
5	Cage Control	0/4	0/7	0	0

Table 2 lists the compound related deaths by group and the percent mortality for the acute oral LD₅₀ study (GLP Study Number 83004) of the DMSO Recycle Solvent (TP013).

Table 2

DMSO Recycle Solvent (TP013)

Compound Related Deaths by Group
Oral Toxicity (LD₅₀), Study 83004

Group	Dose Level	Compound Related Deaths/No. in Group		Percent Mortality	
		Male	Female	Male	Female
1	Cage Control	0/8	0/8	0	0
2	Vehicle Control (Reagent Grade DMSO)	0/8	0/8	0	0
3	0.8 ml/kg	0/8	1/8	0	13
4	1.6 ml/kg	2/8	5/8	25	63
5	3.1 ml/kg	5/6*	8/8	83	100
6	4/7 ml/kg	8/8	7/7*	100	100

*Fewer animals per dose group due to improper administration of the dosing material.

Lethal Dose Calculations

Lethal Dose (LD) values calculated by probit analysis for the DMSO Recycle Solvent (TP013) are presented in Table 3.

Table 3*

Lethal Dose (LD) Levels
For the DMSO Recycle Solvent in Male and Female Rats

Percent Population	Lethal Dose ml/kg		95 Percent Confidence Limits (ml/kg)	
	Male	Female	Male	Female
LD 1	0.9	0.5	(0.3, 2.4)	(0.2, 1.6)
LD 50	2.1	1.3	(1.5, 2.8)	(1.0, 1.8)
LD 95	3.7	2.6	(1.8, 7.4)	(1.2, 5.4)

*Statistician's Report (Appendix E).

Clinical Observations

Animals were observed daily, undisturbed in cages, outside of cages, and after replacement in cages. On the day of dosing, the animals were observed intermittently throughout the entire dosing procedure. Signs of inactivity and sluggishness, interrupted by periods of excitability and irritability, were common findings both in animals that eventually died and those that survived but were affected. The most striking signs ascribed to the dosing material were extensive irritability and excitability in the rats. The affected animals would become highly aggressive when disturbed in cages.

Gross Pathological Observations

The mortalities which occurred after dosing appear to have been caused by the test materials. The dose-response relationship demonstrated with the DMSO Recycle Solvent (TP013) appears to be valid. When surviving animals were sacrificed at conclusion of the study, no test compound-related gross lesions were observed at necropsy. The veterinary pathologist report appears in Appendix F.

DISCUSSION

The DMSO Evaporator Sludge and the Virgin DMSO caused no mortalities at an oral limit dosage of 5.0 ml/kg (5). The DMSO Recycle Solvent produced 100 percent mortalities in both male and female animals at the 5.0 ml/kg oral limit level. The LD₅₀ study was performed with the DMSO Recycle Solvent due to its obvious toxicity at the 5.0 ml/kg oral limit dosage level. The calculated LD₅₀ for the DMSO Recycle Solvent (TP013) in male was 2.1 ml/kg and in female Sprague-Dawley rats was 1.3 ml/kg, with respective 95 percent confidence limits (1.5 ml/kg, 2.8 ml/kg) and (1.0 ml/kg, 1.8 ml/kg).

The predominant clinical signs were depression, inactivity, excitation, and aggression, with a mild to moderate loss of equilibrium.

CONCLUSION

The higher toxicity of the DMSO Recycle Solvent compared to that of the Evaporator Sludge probably is related to the greater amount of RDX and HMX present in solution. The Virgin DMSO sample showed no toxicity. Industrial grade DMSO has a higher percentage of water than reagent grade DMSO (Appendix A); this is the primary difference in the two liquids. The DMSO Recycle Solvent should be classified as moderately toxic (7).

RECOMMENDATION

No recrystallization pilot plant recommendation is warranted solely upon this report. Further data are necessary for the health hazard assessment of the DMSO process stream samples.

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Toxicity Test Sample Composition^a

Concentration by HPLC, g/l

Sample	^b RDX	^c HMX	^d TAX	^e SEX	^g %H ₂ O	%DMSO
Virgin DMSO ^f	0	0	0	0	0.63 ⁱ	99.37 ^j
DMSO Recycle Solvent ^h	24.188	39.542	0.263	0	35.48 ⁱ	58.64 ^j
DMSO Evaporator Sludge ^f	0.548	0.942	3.521	0	5.35 ⁱ	94.19 ^j

Calculated Data In Weight Percent^a

Sample	RDX	HMX	TAX	SEX	H ₂ O	DMSO
Virgin DMSO	0	0	0	0	0.63	99.37
DMSO Recycle Solvent	2.22	3.64	0.02	0	35.48	58.64
DMSO Evaporator Sludge	0.05	0.09	0.32	0	5.35	94.19

^a Data supplied by sponsor^b RDX: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine^c HMX: Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine^d TAX: 1-Acetylhexahydro-3,5-Dinitro-1,3,5-Triazine^e SEX: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine^f At ambient temperature.^g By Karl Fisher^h Analysis of equilibrium liquid at 40 C.ⁱ Water content calculated by difference.^j DMSO content by gas chromatography using Virgin DMSO sample as the standard.

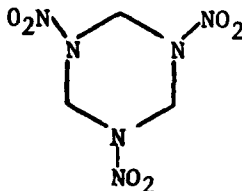
APPENDIX A

Chemical Data

1. Chemical name: Hexahydro-1,3,5-Trinitro-1,3,5-Triazine,
Cyclotrimethylenetrinitramine, Cyclonite
Hexogen, RDX

Chemical Abstract Service Registry Number: 121-82-4

Structural formula:



Empirical formula: C₃H₆N₆O₆

Molecular weight: 222.13 g/mole

Physical State: White crystals varying in size

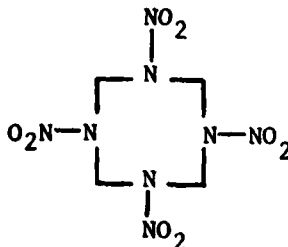
Melting point: 200-203 C

Manufacturer: Holston Army Ammunition Plant
Kingsport, TN

2. Chemical name: Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazine
HMX, Cyclotetramethylenetrinitramine

Chemical Abstract Service Registry Number: 2691-41-0

Structural formula:



Empirical formula: $C_4H_8O_8N_8$

Molecular weight: 296.17 g/mole

Physical state: White crystals of varying size

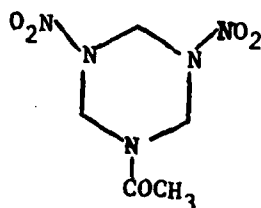
Melting point: 280 C

Manufacturer: Holston Army Ammunition Plant
Kingsport, TN

3. Chemical name: Hexahydro-1-(N)-Acetyl-3,5-Dinitro-1,3,5-Triazine, TAX

Chemical Abstract Service Registry Number: 14168-42-4

Structural formula:



Empirical formula: $C_5H_9O_5N_5$

Molecular weight: 219.17 g/mole

Physical state: White crystals of varying size

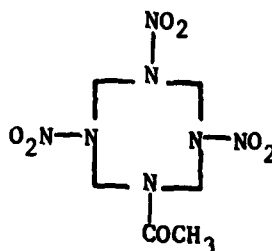
Melting point: 156 C

Manufacturer: By-product of the production/processing of HMX/RDX
at the Holston Army Ammunition Plant, Kingsport, TN

4. Chemical name: Octahydro-1-(N)-Acetyl-3,5,7-Trinitro-1,3,5,7-Tetrazine, SEX

Chemical Abstract Service Registry Number: 13980-00-2

Structural formula:



White--14

Empirical formula: $C_6H_{11}O_7N_7$

Molecular weight: 293.21 g/mole

Physical State: White crystals of varying size

Melting point: 224.2-224.7 C

Manufacturer: By-product of the production/processing of HMX/RDX
at the Army Ammunition Plant, Kingsport, TN

5. Chemical name: Dimethyl Sulfoxide (DMSO)

Chemical Abstract Service Registry Number : 00006-76-85

Structural formula: C_2H_6SO

Empirical structure: $\begin{array}{c} CH_3-S-CH_3 \\ | \quad | \\ \quad O \end{array}$

Molecular weight: 78.02 g/mole

Physical state/color: Clear transparent liquid.

Freezing point: 18.55 C

Boiling point: 189 C

Contaminants: Water 0.63 percent

Manufacturer: Crown Zellerbach Corporation
Chemical Products Division
Camas, WA 98607

6. Chemical name: Dimethyl Sulfoxide (DMSO) reagent grade

Chemical Abstract Service Registry Number: 00006-76-85

Structural formula: $\begin{array}{c} CH_3-S-CH_3 \\ | \quad | \\ \quad O \end{array}$

Empirical formula: C_2H_6SO

Physical state: Clear transparent liquid

Freezing point: 18.3 C

Boiling point: 189 C

Density: 1.095 g/ml

Contaminants: Water 0.08%

Manufacturer: J.T. Baker Chemical Co.
Phillipsburg, NJ 08805

HOLSTON DEFENSE CORPORATION

WEST STONE DRIVE
KINGSPORT, TENNESSEE 37660

June 22, 1983

TELEPHONE: AREA CODE 615 247-9111

Contracting Officer's Representative
Holston Army Ammunition Plant
Kingsport, Tennessee 37660

Dear Sir:

Subject: DMSO Process Stream Toxicological Testing

Reference: USAMBRDL Letter to Commander, HSAAP, "DMSO Munition Process Solvent Toxicology Studies Laboratory Monitoring Visits and Technical Status Review Meetings," dated November 23, 1982

1. The meetings referred to in the above reference were attended as requested. At that time the toxicity studies at both LAIR and LEHR were just getting under way, and the meetings were used to review preliminary results then available as well as plans for completing the studies. Holston was also involved in a characterization screening study of the same test samples in an attempt to identify potentially toxic compounds which might be present and could contribute to the toxic or mutagenic results observed.

The test samples had been previously analyzed for composition at Holston and shipped to LAIR. At the referenced meeting, Col. Fruin requested that in addition Holston furnish both the results of the characterization screening study and the details of the analytical methods used to perform the original quantitative analyses on the test samples at Holston. The screening study at Holston has now been completed, and the requested information is hereby transmitted.

2. The characterization screening study was performed on the composite recycle solvent sample from the DMSO pilot plant. Also, production crude/water-washed RDX and HMX samples were subjected to analyses to determine if any unusual compounds could be detected for comparison with any found in the DMSO sample. HPLC methods were used during the screening procedure varying the columns, solvent systems, wavelengths, and the other parameters such that any contaminant peaks found could be identified by component retention time.

Initial HPLC analysis of the recycle solvent sample showed very large concentrations of RDX and HMX which interfered with analysis of other components. The sample was treated to remove the bulk of the RDX and HMX by heating to 40°C and then quenching one to one with water. The decanted liquid was then subjected to the remainder of the screening

APPENDIX A (cont.)

Contracting Officer's Representative
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study analyses. The sample was examined by several HPLC systems available at Holston which are normally used to analyze RDX, HMX, and related nitramines found in various plant process streams and products. These are presented in Attachments II and III. Other HPLC conditions presented in Attachment I, which do not represent proven HPLC methods, were also used to get as much system variability as possible. Note that Holston does not guarantee these results since these procedures in Attachment I were used only for screening and qualitative purposes. It should also be realized that most of Holston's routine procedures are used to detect nitramine or related compounds. Other impurities may not have been detected by these methods. The only compounds detected using any of the systems were RDX, HMX, SEX, and TAX. HPLC retention times for these compounds matched the known retention times for RDX, HMX, SEX, and TAX. Attachment I also presents the results obtained. Analysis of crude RDX and HMX by the methods described in Attachment II yielded no evidence of the presence of compounds other than RDX, HMX, and SEX.

3. Quantitative analyses of the test samples were performed by HPLC. Since no reliable method for direct analysis of DMSO by either HPLC or GC has been developed, DMSO values are by difference. Attachment III presents an outline of the quantitative methods used.
4. This information should be transmitted to the following:

Col. John Fruin
Building 1110
Presidio of San Francisco
California 94129

Capt. James Carroll
USAMBRDL
Building 568
Fort Detrick
Frederick, Maryland 21701

Raymond Goldstein
ARRADCOM
Picatinny Arsenal
Dover, New Jersey

Yours very truly,

HOLSTON DEFENSE CORPORATION


M B Knowles
Plant Manager

Attachments (3)

APPENDIX A (cont.)

<u>HPLC Parameters</u>	<u>Components Detected</u>
1. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 NM Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX SEX
2. Column: LiChrosorb-Amine, 1/4" x 12" ss Detector: UV, 230-260 nm in 10 nm increments Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX
3. Column: LiChrosorb-Diol, 1/4" x 12" ss Detector: UV, 230-260 nm in 10 nm increments Solvent System: 70% iso-octane 15% chloroform 10% acetonitrile 5% methanol Flow Rate: 3.0 ml/min Injection Volume: 10 microliters	RDX HMX
4. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 70% water 30% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX
5. Column: Waters CN, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX
6. Column: Waters CN, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 60% water 40% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX

HPLC Parameters	Components Detected
7. Column: Waters CN, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 50% water 50% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
8. Column: LiChrosorb-Diol, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
9. Column: LiChrosorb-Amine, 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
10. Column: LiChrosorb-RP18, 1/4" x 12" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	RDX HMX TAX SEX
11. Column: LiChrosorb-RP18 1/4" x 12" ss Detector: UV at 254 nm Solvent System: 60% water 40% methanol Flow Rate: 2.5 ml/min Injection Volume: 10 microliters	No component separation
12. Column: LiChrosorb-RP 8 1/4" x 6" ss Detector: UV, 215-290 nm in 10 nm increments Solvent System: 80% water 20% methanol Flow Rate: 2.0 ml/min Injection Volume: 10 microliters	RDX HMX TAX SEX
13. Column: LiChrosorb-RP 8 1/4" x 6" ss Detector: UV at 254 nm Solvent System: 60% water 40% methanol Flow Rate: 2.0 ml/min Injection Volume: 10 microliters	No component separation

ATTACHMENT II

HPLC Analysis of Crude RDXHPLC Parameters

Column: Waters CN, 1/4" x 12" ss
Detector: UV, 215-290 nm in
10 nm increments
Solvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol
Flow Rate: 3.0 ml/min
Injection Volume: 10 microliters

Components Detected

RDX
HMX
SEX

HPLC Analysis of Crude HMXHPLC Parameters

Column: Waters CN, 1/4" x 12" ss
Detector: UV, 215-290 nm in
10 nm increments
Solvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol
Flow Rate: 3.0 ml/min
Injection Volume: 10 microliters

Components Detected

RDX
HMX
SEX

ATTACHMENT III

Quantitative Analysis of DMSO/Explosives SamplesSample Preparation

1. Weigh representative liquid sample.
2. Evaporate sample to dryness - weigh dried sample.
3. Add acetonitrile to sample sufficient to completely dissolve all solids.
4. Analyze for RDX, HMX, and SEX using Procedure A below.
5. Analyze for TAX using Procedure B below.

Procedure A - HPLC

Column: Waters CN, 1/4" x 12" ss (Waters No. 84082)

Detector: UV at 254 nm

Solvent System: 70% iso-octane
15% chloroform
10% acetonitrile
5% methanol

Flow Rate: 3.0 ml/min

Injection Volume: 10 microliters

Typical Retention Times (seconds): RDX - 195
SEX - 365
HMX - 423

Procedure B - HPLC

Column: Waters CN, 1/4" x 12" ss (Waters No. 84082)

Detector: UV at 254 nm

Solvent System: 80% water
20% methanol

Flow Rate: 2.5 ml/min

Injection Volume: 10 microliters

DMSO/Water Content

Karl Fischer titration was used to determine the water content of the liquid recycle solvent. DMSO was determined by difference as below:

$$\% \text{ DMSO} = 100\% - \% \text{ Solids} - \% \text{ Water}$$

ANIMAL DATA
GLP STUDY 82039

Study Type: Acute Oral Toxicity Limit Test in Male and Female Rats of
DMSO Process Stream Samples.

Species: Albino laboratory rat (Rattus norvegicus)

Strain: Sprague-Dawley

Source: Banton Kingman
Fremont, CA

Sex: Male and Female

Age: On Day of Dosing, Male 6 weeks
Female 7 weeks

Dates of Birth: Male - 24 November 1982
Female - 16 November 1982

Method of Randomization: Weigh bias stratified using the RANDOM
Computer Program (LAIR SOP OP-156-21).

Animals in Each Group: 7 male and 7 female animals.

Condition of Animals at Start of Study: Normal

Body Weight Range at Dosing: Males 163 to 234 g
Females 167 to 203 g

Identification Procedures: Ear tagged (LAIR SOP OP-ARG-1), numbers
82D01226 to 82D01294, with no exclusions.

Pretest Conditioning: Quarantine/acclimation 29 December 1982 to 4
January 1983

Justification: The laboratory rat has been proven to be a sensitive
and reliable system for lethal dose determination.

ANIMAL DATA
GLP STUDY 83004

Study Type: Acute Oral Toxicity (LD_{50}) Test in Male and Female Rats
of DMSO Process Stream Sample Recycle Solvent (TP013)

Species: Albino laboratory rat (Rattus norvegicus)

Strain: Sprague-Dawley

Source: Banton Kingman
Fremont, CA

Sex: Male and Female

Age: On day of dosing (23 March 1983)
Male 6 weeks
Female 7 weeks

Dates of Birth: Male 10 February 1983
Female 3 February 1983

Method of Randomization: Weight bias stratified using the RANDOM
Computer Program (LAIR SOP OP-ISG-21).

Animals in Each Group: 8 males, 8 females

Condition of Animals at Start of Study: Normal.

Body Weight Range at Dosing: Males 216-269 g
Females 169-215 g

Identification Procedure: Ear tagged (LAIR SOP OP-ARG-1), numbers
83D00173 to 3D00278 without exclusions.

Pretest Conditioning: Quarantine/acclimation 17-22 March 1983.

Justification: The laboratory rat has been proven to be a sensitive
and reliable system for lethal dose determination

ENVIRONMENTAL CONDITIONS

Caging: Number/cage - 1

Type of Cages Used: Stainless steel "mesh drawer rack" type. Bedding none.

Diet: Certified Purina Rodent Chow, Diet #5002
Ralston Purina Company
Checkerboard Square
St. Louis, MO 63188
ad lib.

Water: Automatic Lixit dispenser.

Temperature: 21-22^C

Relative Humidity: 40 \pm 5%

Photoperiod: 0530 to 2000 hours per day (light 14-1/2 hours).

HISTORICAL LISTING OF STUDY EVENTS
GLP STUDY 82039

<u>Date</u>	<u>Event</u>
29 Dec 82	Animals arrived at LAIR. Sex was determined; they were observed for illness, ear tagged, weighed, and caged in the GLP Suite.
30 Dec 82 4 Jan 83	Animals checked once daily.
3 Jan 83	Animals weighed and randomized into dose groups.
30 Dec 82, 3 Jan 83	Animals weighed.
3, 4 Jan 83	Animals conditioned to dosing procedure using sterile gavage tubes (no material injected).
5 Jan 83	Animals weighed and dosed. Observations conducted intermittently after dosing and during the afternoon. Animals observed for clinical signs which were recorded.
6-18 Jan 83	Animals observed daily for clinical signs at 1300 hours.
7,10,14 Jan 83	All animals weighed.
18 Jan 83	Food removed at 1500 hours.
19 Jan 83	Animals observed for clinical signs at 0700 hours and weighed. Animals delivered to the PSG Necropsy Suite for sacrifice and gross necropsy by 0900 hours.

APPENDIX D

HISTORICAL LISTING OF STUDY EVENTS
GLP STUDY 83004

16 Mar 83	Animals arrived at LAIR. Sex was determined; they were observed for illness, ear tagged, weighed, and caged in GLP Suite.
17-22 Mar 83	Animals checked once daily.
18 Mar 83	Animals weighed and randomized into dose groups.
21 Mar 83	Animals weighed.
21, 22 Mar 83	Animals conditioned to dosing procedure using sterile gavage tubes (no material injected).
23 Mar 83	Animals weighed and dosed. Observations conducted and recorded.
24 Mar - 4 Apr 83	Animals observed daily for clinical signs.
25, 28 Mar-1 Apr 83	All animals weighed.
4 Apr 83	Food removed at 1600 hours.
5 Apr 83	Animals observed for clinical signs at 0700 hours and weighed. Animals delivered to the PSG Necropsy Suite for sacrifice and gross necropsy by 0900 hours.

APPENDIX D (concluded)

STATISTICAL ANALYSIS

Eight male and eight female animals were assigned to each of six dose groups by simple random sampling techniques using a program, Random, on the Data General C 330 Computer.

Bliss method of probit analysis was used to determine the LD₁, LD₅₀, and LD₉₅ values along with the corresponding 95% confidence limits. The program, PROBIT, was used to determine the probit curve and the lethal dose values. The probit regression line fit to the data was:

$$\begin{aligned} \text{Males} & - Y = 2.9 + 6.5 \text{ Log } X \\ \text{Females} & - Y = 4.3 + 5.8 \text{ Log } X \end{aligned}$$

where X is the dose and Y the corresponding probit value.

Virginia L. Gildengorin
 VIRGINIA L. GILDENGORIN, PhD
 DAC, Statistician
 18 August 1983

* Please refer to Table 3 in body of report, page 6.

PATHOLOGY REPORT

GLP Study 82039

Acute Oral Toxicity Limit Test in Male Rats of: DMSO Process
Stream Sampler [DMSO Recycle Solvent (TPO13), Virgin DMSO Solution
(TPO14), and DMSO Evaporator Sludge (TPO15)]

History: The male Sprague-Dawley rats in this study were divided into 5 groups. All groups but the cage controls received a single dose of either 5 ml/kg of vehicle or one of the test solutions by oral gavage. The solution dosed with and number of rats in each group were as follow:

(Cage controls) - 4 rats
Group 1 (Vehicle controls) - 7 rats
Group 2 (TPO13) - 7 rats
Group 3 (TPO14) - 7 rats
Group 4 (TPO15) - 7 rats

Seven of 7 rats in group 2 were found dead between 2 hours and 37 minutes and 123 hours and 34 minutes after being dosed with DMSO Recycle Solvent (TPO13). All other rats survived until termination of the study, 14 days after dosing. The rats were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Gross necropsy findings: All of the rats in group 2 died and had gross lesions [as follow] that were due to the tested solution: the lungs of 7/7 rats were mottled or diffusely red to black, were wet, and failed to collapse, the large pulmonary airways of 2/7 rats contained blood and 1/7 contained clear froth, the rat with the clear pulmonary froth had hydrothorax, and the muzzle of 6/7 rats was covered with bloody material.

Two of 7 rats in group 2, that were found dead less than 4 hours after dosing, had thin white flocculent material in their stomachs that was probably test solution and mucus. Four of 7 rats in group 2 were mildly autolyzed.

Necropsies revealed no test compound-related gross lesions in male rats that were killed at termination of the study. One of 4 cage controls had a focal skin abrasion on its shoulder that was considered to be an incidental finding.

Summary:

1. Seven of 7 male Sprague-Dawley rats dosed with 5 ml/kg of DMSO Recycle Solvent (TPO13) died and had prominent gross respiratory lesions that were due to the test solution.

2. No test-related gross lesions were observed in male Sprague-Dawley rats that were cage controls, vehicle controls, dosed with 5 ml/kg of Virgin DMSO (TPO14), or dosed with 5 ml/kg of Evaporatory Sludge (TPO15).

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS

Diplomate, A.C.V.P.

MAJ, VC

Assistant Chief, Pathology Services Group

Division of Research Support

28 February 1983

PATHOLOGY REPORT

GLP Study 82039

Acute Oral Toxicity Limit Test in Female Rats of: DMSO Process
Stream Sampler [DMSO Recycle Solvent (TPO13), Virgin DMSO Solution
(TPO14), and DMSO Evaporator Sludge (TPO15)]

History: The female Sprague-Dawley rats in this study were divided into 5 groups. All groups but the cage controls received a single dose of either 5 ml/kg of vehicle or one of the test solutions by oral gavage. The solution dosed with and number of rats in each group were as follow:

- (Cage controls) - 7 rats
- Group 1 (Vehicle controls) - 7 rats
- Group 2 (TPO13) - 7 rats
- Group 3 (TPO14) - 7 rats
- Group 4 (TPO15) - 7 rats

Seven of 7 rats in group 2 were found dead between 3 hours and 36 minutes and 140 hours and 50 minutes after being dosed with DMSO Recycle Solvent (TPO13). All other rats survived until termination of the study, 14 days after dosing. The rats were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Gross necropsy findings: All of the rats in group 2 died and had gross lesions as follow that were due to the tested solution: the lungs of 7/7 rats were mottled or diffusely red to black, were wet, and failed to collapse, their muzzles were covered with red material, and the large pulmonary airways of 5/7 rats contained blood.

The kidneys of 1/7 rats in group 2 were swollen and had a dark zone at the corticomedullary junction that may have been due to the test solution.

Five of 7 rats in group 2, that were found dead less than 21 hours after dosing, had thin white flocculent material in their stomachs that was probably test solution and mucus. Seven of 7 rats in grup 2 were mildly autolyzed.

Necropsies revealed no test compound related gross lesions in female rats that were killed at termination of the study. One of 7 rats in group 1 had a solitary cortical cyst in one kidney and 1/7 rats in group 4 had bilateral hydronephrosis and a solitary cortical cyst that contained yellow crystals in 1 kidney. The renal lesions were considered to be incidental findings.

APPENDIX F (cont.)

Summary:

1. Seven of 7 female Sprague-Dawley rats dosed with 5 ml/kg of DMSO Recycle Solvent (TPO13) died and had prominent gross respiratory lesions that were due to the tested solution.
2. The swollen kidneys with a dark zone at the corticomedullary junction in 1/7 rats dosed with 5 ml/kg of TPO13 may have been due to the tested solution.
3. No test-related gross lesions were observed female Sprague-Dawley rats that were cage controls, vehicle controls, dosed with 5 ml/kg of Virgin DMSO (TPO14), or dosed with 5 ml/kg of Evaporatory Sludge (TPO15).

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS

Diplomate, A.C.V.P.

MAJ, VC

Assistant Chief, Pathology Services Group

Division of Research Support

28 February 1983

PATHOLOGY REPORT

GLP Study 83004

Acute Oral Toxicity Study (LD₅₀) of DMSO Process Stream Recycle Solvent Sample (LAIR Code No. TPO13) in Male Rats

History: The male Sprague-Dawley rats in this study were divided into 6 groups; a cage control group, a vehicle control group, and 4 dose groups. All groups but the cage controls received a dose of either 400 mg/kg reagent grade DMSO (vehicle controls) or DMSO Process Stream Recycle Solvent solution (TPO13) as total nitramines in mg/kg by oral gavage. The solution dosed with and number of rats in each group were as follow:

Group 1 (Cage controls) - 8 rats

Group 2 (Vehicle controls) - 8 rats

Group 3 [50 mg/kg (0.8 ml/kg)] - 8 rats

Group 4 [100 mg/kg (1.6 ml/kg)] - 8 rats

Group 5 [200 mg/kg (3.1 ml/kg)] - 6 rats

Group 6 [300 mg/kg (4.7 ml/kg)] - 8 rats

Fifteen rats were found dead between 6 hours and 34 minutes and 118 hours and 44 minutes after being dosed with TPO13. The deaths of 2/8* rats in group 4, 5/6 rats in group 5, and 8/8 rats in group 6 were due to the TPO13 solutions and a dose-response was indicated. All other rats survived until the conclusion of the study. The surviving rats were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Necropsy findings: All of the 15 rats that died had gross lesions that were due to the tested solutions. The lungs of 2/2 rats in group 4, 2/5 rats in group 5, and 4/8 rats in group 6 were mottled red and the trachea of 1/5 rats in group 5 contained a large amount of froth. Blood-tinged wet or crusty material was present on the muzzle of 2/2 rats in group 4, 4/5 rats in group 5, and 8/8 rats in group 6. Paraphimosis was present in 1/2 rats in group 4, 3/5 rats in group 5, and 2/8 rats in group 6. Focal necrotizing dermatitis was present on the scrotum of 1/2 rats in group 4, 4/5 rats in group 5 and 5/8 rats in group 6. The scrotal lesion that was diagnosed as necrotizing dermatitis may have been diapedesis and the exudation of plasma.

*Number of rats affected/Number of rats in the group or Number of rats affected/Number of rats that died.

The following lesions were present in 3/15 rats that died and may have been due to the tested solutions: engorged meningeal vessels were present in the cranium of 1/2 rats in group 4 and 1/5 rats in group 5, and mucosal petechia were present in the stomach of 1/8 rats in group 6.

Necropsies revealed no test solution related gross lesions in the male Sprague-Dawley rats that were killed at the conclusion of the study. One of 8 rats in group 3 had a kidney with a dilated pelvis that was considered to be an incidental finding.

Summary:

1. The deaths of 15 male rats were due to the tested solutions and a dose response was indicated.
2. One or more of the gross lesions in the lungs and trachea, around the muzzle, and of the scrotum and penis observed in the rats that died were due to tested solutions. The lesions may have been due to altered vascular integrity.
3. No gross lesions, related to the tested solutions, were observed in the male rats that were killed at the conclusion of the study.

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS

Diplomate, A.C.V.P.

MAJ, VC

Assistant Chief, Pathology Services Group
Division of Research Support

9 June 1983

PATHOLOGY REPORT

GLP Study 83004

Acute Oral Toxicity Study (LD_{50}) of DMSO Process Stream Recycle Solvent Sample (LAIR Code No. TPO13) in Female Rats

History: The female Sprague-Dawley rats in this study were divided into 6 groups; a cage control group, a vehicle control group, and 4 dose groups. All groups but the cage controls received a dose of either 400 mg/kg reagent grade DMSO (vehicle controls) or DMSO Process Stream Recycle Solvent solution (TPO13) as total nitramines in mg/kg by oral gavage. The solution dosed with and number of rats in each group were as follow:

- Group 1 (Cage controls) - 8 rats
- Group 2 (Vehicle controls) - 8 rats
- Group 3 [50 mg/kg (0.8 ml/kg)] - 8 rats
- Group 4 [100 mg/kg (1.6 ml/kg)] - 8 rats
- Group 5 [200 mg/kg (3.1 ml/kg)] - 8 rats
- Group 6 [300 mg/kg (4.7 ml/kg)] - 7 rats

Twenty-one rats were found dead between 4 hours and 36 minutes and 30 hours and 18 minutes after being dosed with TPO13. The deaths of 1/8* rats in group 3, 5/8 rats in group 4, 8/8 rats in group 5, 7/7 rats in group 6 were due to the TPO13 solutions and a dose-response was indicated. All other rats survived until the conclusion of the study. The surviving rats were killed by exsanguination from severed axillary vessels while under anesthesia produced by intraperitoneal injection of pentobarbital.

Gross necropsy findings: Twenty of the 21 rats that died had gross lesions that were due to the tested solutions. The lungs of 1/1 rats in group 3, 3/5 rats in group 4, 3/8 rats in group 5, and 4/7 rats in group 6 were mottled dark red. Blood-tinged wet, mucoid, or crusty material was present on the muzzle of 1/1 rats in group 3, 5/5 rats in group 4, 8/8 rats in group 5, and 6/7 rats in group 6. Blood-tinged wet, mucoid, or crusty material was present in the mouth, on the forepaws, or on the ventral surface of 2/5 rats in group 4, 3/8 rats in group 5, and 3/7 rats in group 6.

*Number of rats affected/Number of rats in the group or Number of rats affected/Number of rats that died

The following lesions were present in 4/21 rats that died and may have been due to the tested solutions: engorged meningeal vessels were present in the cranium of 1/5 rats in group 4 and 2/8 rats in group 5, ventricular hemorrhage in the brain was present in 1/8 rats in group 5, and mucosal petechia were present in the stomach of 1/5 rats in group 4.

One of 5 rats in group 4 and 2/8 rats in group 5 were autolyzed. One of the 7 rats in group 6 that died had no gross lesions.

Necropsies revealed no test solution related gross lesions in the female Sprague-Dawley rats that were killed at the conclusion of the study.

Summary:

1. The deaths of 21 female rats were due to the tested solutions and a dose response was indicated.
2. Gross lesions in the lungs and/or blood tinged material from the respiratory and/or upper digestive tract due to the tested solutions were present in all but one of the rats that died. The lesions may have been due to altered vascular integrity.
3. Female Sprague-Dawley rats may be more sensitive than male rats to the toxic effect of TPO13.
4. No gross lesions were observed in the female rats that were killed at the conclusion of the study.

Glen E. Marrs, Jr.

GLEN E. MARRS, JR., DVM, MS
Diplomate, A.C.V.P.
MAJ, VC
Assistant Chief, Pathology Services Group
Division of Research Support

9 June 1983

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